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Rabbit Liver Metallothionein Tentative Amino Acid Sequence of Metallothionein-B*

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The primary structure of equine renal metallothionein-1B was first reported by Kojima *et al.* Most recently, Kissling and Kägi have elucidated the amino acid sequence of human hepatic metallothionein-II and compared it to the known structure of the former. Huang *et al.* have also demonstrated the complete sequence of hepatic metallothionein-I obtained from the liver of mouse administered with cadmium. We report the amino acid sequence of liver metallothionein-B isolated from rabbits exposed to cadmium.

Rabbit liver metallothionein-A and -B were isolated from rabbits exposed to cadmium (by 21 subcutaneous injections of 1 mg/kg body weight with intervals of 2—3 days). On the second day after the last injection the animals were killed. To one volume of the liver homogenate in 0.02 M Tris-HCl buffer, pH 8.6, cold ethanol and chloroform (1.00: 1.05: 0.08, by volume) were added with cooling. To the supernatant obtained by centrifugation three volumes of cold ethanol were added subsequently. The solution was allowed to stand at -20°C overnight. The produced precipitate was collected by centrifugation. It was dissolved in 0.02 M ammonium carbonate and then it was charged to a Sephadex G-75 column. The elution was carried out by use of 0.02 M ammonium carbonate. The Cd-binding protein fraction of low molecular weight on the gel filtration was further separated into the Cd-binding proteins, metallothionein-fA, a minor component, -A and -B, major components, by ion exchange chromatography on a DEAE Sephadex A-25 column.

Metallothionein was S-pyridylethylated with 4-vinylpyridine at pH 8.0 by using the modified method of Friedman *et al.* and S- ^{14}C -carboxymethylated with iodoacetic acid-2- ^{14}C at pH 8.0 by using the modified method of Crestfield *et al.* after the removal of metals by the treatment of 6 M guanidine HCl in 0.2 N HCl. Acid hydrolysates of the intact protein and the oxidized one with performic acid were analyzed by an amino acid analyzer. S- ^{14}C -carboxymethylated metallothionein was digested with trypsin. The tryptic peptides were separated by ion exchange chromatography on a Dowex-50 ($\times 2$) column with gradient elution of pyridine-acetate buffer. Further purification of peptide was carried out by use of paper electrophoresis and/or paper chromatography. The fragment used for the automated sequence analysis was obtained by cyanogen bromide cleavage and by selective cleavage of labile bond between Asp-Pro with 70% formic acid. The fragments were

* *Metallothionein* J.H.R. Kägi and M. Nordberg (eds) Birkhäuser Verlag, Basel/Boston/Stuttgart, 1979, pp. 163~168.

thionein molecules, as pointed out in the other report, described by Kimura *et al.*. However, the chemical structure of metallothionein obtained from the animals exposed to cadmium is as a whole similar to that from the intact animal and human being, as illustrated by the present report and the other papers described by Huang *et al.*